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14. ABSTRACT

The molecular and cellular origins of prostate cancer are poorly understood. Recent evidence from our laboratory suggests that prostate cancer may arise from a basal/luminal precursor cell marked by cell surface expression of PSCA. The evidence supporting this hypothesis is that (1) PSCA marks an intermediate cell population that coexpresses basal and luminal cell cytokeratins (2) this cell population is does not express p63 and is androgen receptor positive, all hallmarks of prostate cancer, and (3) PSCA is highly expressed in HGPIN and prostate cancer and in all animal models of prostate cancer. To test this hypothesis and to develop new models of prostate, we propose to determine whether delivery of oncogenes specifically to the PSCA positive cells of mouse prostate is sufficient to cause cancer. To accomplish this, we will develop a transgenic mouse model in which the retroviral receptor gene tva is expressed in the prostate under control of the PSCA promoter. Virus containing one or more oncogenes will be delivered to the prostate and the resulting phenotype characterized.

15. SUBJECT TERMS
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INTRODUCTION

The molecular and cellular origins of prostate cancer are poorly understood. Recent evidence from our laboratory suggests that prostate cancer may arise from a basal/luminal precursor cell marked by cell surface expression of PSCA. The evidence supporting this hypothesis is that (1) PSCA marks an intermediate cell population that co-expresses basal and luminal cell cytokeratins (2) this cell population is does not express p63 and is androgen receptor positive, all hallmarks of prostate cancer, and (3) PSCA is highly expressed in HGPIN and prostate cancer and in all animal models of prostate cancer. To test this hypothesis and to develop new models of prostate, we propose to determine whether delivery of oncogenes specifically to the PSCA positive cells of mouse prostate is sufficient to cause cancer. To accomplish this, we develop a transgenic mouse model in which the retroviral receptor gene tva is expressed in the prostate under control of the PSCA promoter. Virus containing one or more oncogenes will be delivered to the prostate and the resulting phenotype characterized.

FINAL REPORT

Specific Aim 1. Establishment of a PSCA-tva transgenic line and characterization of the TVA positive cell population. (months 1-18). This aim includes the following tasks:

1. establishment of transgenic lines (done) and breeding and expansion of line, including derivation of homozygous offspring (months 1-6)
2. optimization and standardization of RCAS-GFP infection with respect to mode of delivery, dosing and time of delivery (puberty vs. post-castration vs. adulthood) (months 1-12)
3. characterization of GFP positive population *in vivo* (months 12-18)
4. characterization of GFP positive population *in vitro*, with standardization of *in vitro* conditions for prostate epithelial growth (months 6-18)

Task 1. Multiple PSCA-tva transgenic lines were established. Expression of TVA was confirmed in the prostates of all lines (Figure 1). TVA expression was confirmed in the prostate at the RNA and protein levels. As predicted, expression was also detected in bladder and stomach. Two transgenic lines were maintained and expanded for *in vivo* orthotopic experiments.

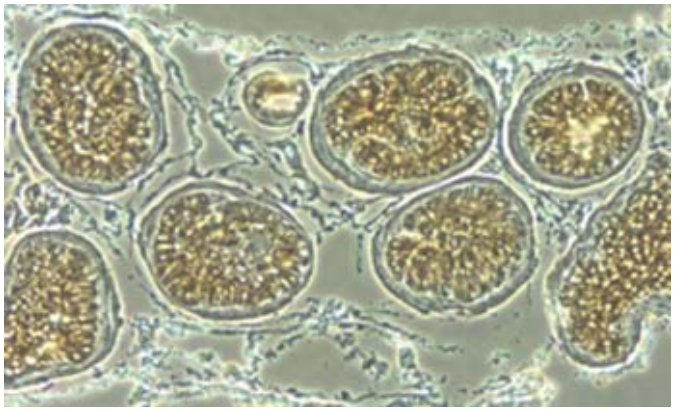

Figure 1: TVA expression in dorsal lobe of mouse prostate. Note epithelial expression. No detectable expression is noted in surrounding stroma.

Task 2. Orthotopic delivery of virus was optimized and standardized, first using the marker gene GFP, and subsequently using luciferase. The latter marker enables us to monitor gene uptake into the prostate noninvasively by imaging with the CCD camera. Orthotopic injection of virus into the dorsal lobe at 5 weeks of age resulted in reproducible uptake of virus and luciferase expression (Figure 2). Control mice were negative.

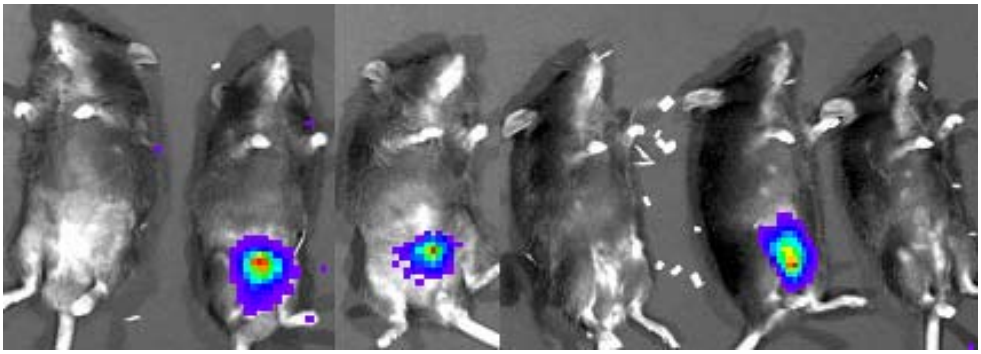

Figure 2: Expression of luciferase in the prostates of 3 PSCA-tva transgenic mice compared with 3 wild-type mice. All were injected with virus at 5 weeks of age orthotopically into the prostate and imaged with the CCD camera at 3 weeks later.

Additional experiments were performed to determine if systemic administration of virus by intraperitoneal injection (IP) could improve prostatic uptake, since a limitation of the orthotopic approach is that virus uptake in the lateral and ventral lobes, which express high levels of TVA, is suboptimal. IP injection of luciferase-expressing virus into five days old pups resulted in significant expression of luciferase in the pelvic and stomach regions. When mice reached puberty at 4 weeks of ages, luciferase expression was absent in the stomach but was maintained in the pelvic area, however the signal continued to decrease and was not observed beyond 8 weeks. In a parallel experiment, middle T virus were delivered via IP and mice sacrificed 3 months later, middle T expression were clearly detected by in situ hybridization in the prostate and stomach. Based on this result, we combined both IP and orthotopic deliveries in our subsequent experiments.

Task 3 - 4. The PSCA/TVA positive cells were further characterized to determine if they are equivalent to the human PSCA-positive prostate epithelial cells (i.e intermediate cells). Two approaches were used. First, we tried to simply monitor the fate of GFP injected cells *in vivo*. This was unsuccessful, as the percentage of cells expressing GFP after orthotopic injection was too low to enable us to follow them over time. Second, we worked out conditions to culture the TVA positive cells *in vitro* and then performed similar analyses to those done with human cells. These studies showed that the mouse TVA positive cells were the same as the PSCA-positive human ones. TVA was expressed by only a subpopulation of mouse prostate epithelium in culture (figure 3). The percentage of TVA positive cells increased with passaging. They were phenotypically distinct from other cells and appeared to form a second layer above the monolayer (figure 3). These were hallmarks of the human cells.

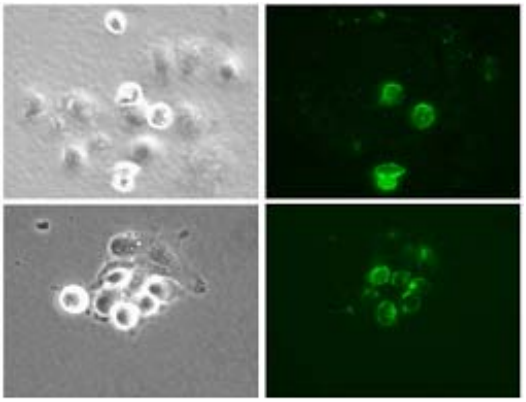

Figure 3: Mouse prostate epithelial cells in tissue culture (left). Immunofluorescent staining of TVA positive cells (green cells on right). Note that TVA positive cells are a subpopulation of the total cellular pool. Also note that the green cells represent those cells on the left, which appear more white and occupy a more superficial cell layer. These are hallmarks of PSCA cells in human prostate cultures as well.

Finally, we performed a recombination experiment, in which the PSCA-tva mouse prostate cells were mixed with mesenchymal cells isolated from the mouse urogenital sinus, and inoculated under the kidney capsule of immunodeficient mice. The reconstituted tissue harvested at 8 weeks later, showed prostate gland-like structures. When we looked for TVA expression by immunohistochemistry in the glands, higher signal was observed in what appeared to be developing gland while cells lining the mature glands had lower to negligible level of expression (Figure 4). This is consistent with the hypothesis that PSCA-tva is a marker of an intermediate precursor cell in the developing prostate. We proceeded to Aim 2 to determine if this cell type is transformable in this model.

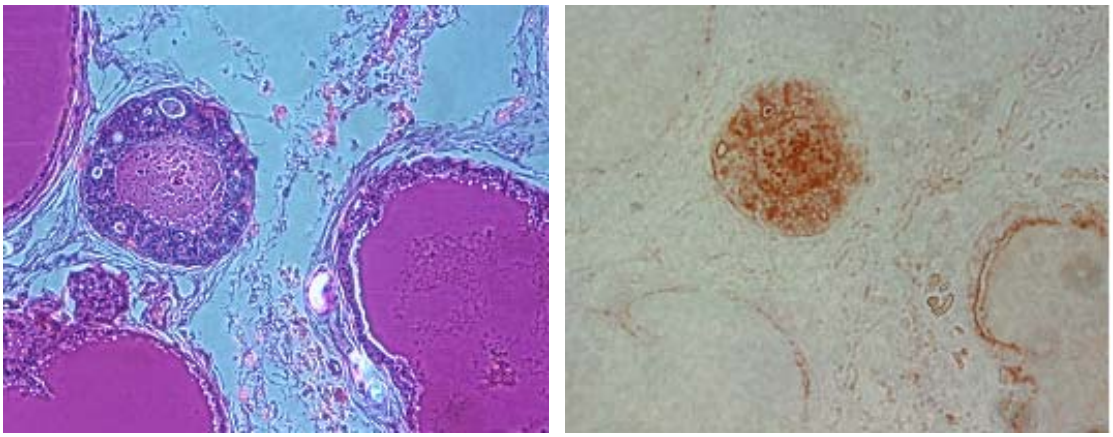

Figure 4: TVA expression in recombinant tissue from PSCA-tva mouse. H/E staining and immunohistochemistry of reconstituted prostate gland-like structure (x 400).

Specific Aim 2. Induction of cancer using the oncogene polyoma virus middle T antigen (months 12-36). This aim includes the following tasks:

1. *in vivo* infection of middle T antigen in mice at puberty or postcastration and monitoring for tumor formation (months 12-24)
2. *in vivo* infection of adult mice to better resemble human prostate cancer (months 18-30)
3. characterization of tumors looking at downstream signaling pathways etc. (months 16-36)
4. crossing of PSCA-TVA and $Nkx3.1^{-/-}$ or $p27^{-/-}$ mice for future experiments in which activated Akt virus will be introduced into the double transgenic prostates (months 24-36 if time permited)

Task 1-3. Middle T antigen virus was delivered into more than 10 transgenic prostates. By RNA *in situ* hybridization middle T was shown to be expressed by a subset of cells as expected. At 6 months after virus injection, prostates of these mice were harvested. Unexpectedly we found that 50 % of mice developed large hemangiomas (Figure 5). Parallel experiments with middle T injection into bladder resulted in the same large hemangiomas. It turns out that endothelial cells are exquisitely sensitive to transformation by middle T antigen leading to hemangioma formation. Although there is no detectable TVA expression whatsoever in the endothelium, it seems likely that the inflammation or trauma of orthotopic injection or very low-level expression of PSCA/TVA by prostatic endothelium resulted in hemangioma formation. We performed *in situ* hybridization on these tissues, and found middle T mRNA expression also in the apparently normal prostate glands adjacent to the hemangioma (Figure 6). We also examined mice harvested at 10 months post middle T injection and found similar results, in that hemangioma were present in some mice, but the epithelial glands were unaffected even though middle T was expressed. This result demonstrated that while middle T can affect endothelial cells dramatically, its expression alone appeared insufficient to cause abnormality in the prostate

epithelium. However, it is evident that genes delivered by this system can be stably expressed in long term (6 - 10 months post injection), even though the co-injected luciferase signal was no longer observed.

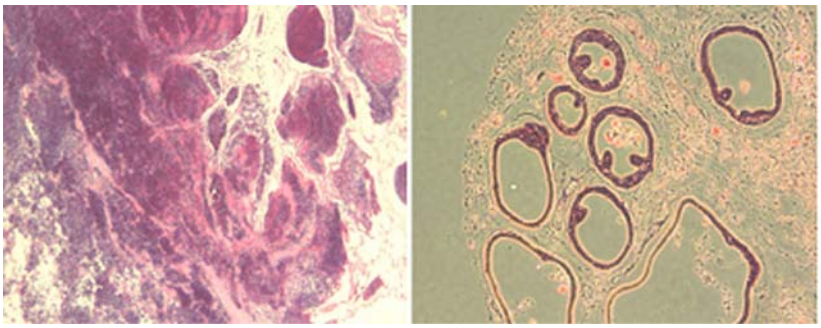

Figure 5: Histology of PSCA-tva mouse prostate injected with middle T antigen, sacrificed at 6 months. Large hemangioma was presented around and within the dorsal prostate (left panel), while remnants of normal prostate glands were also observed in adjacent area (right panel).

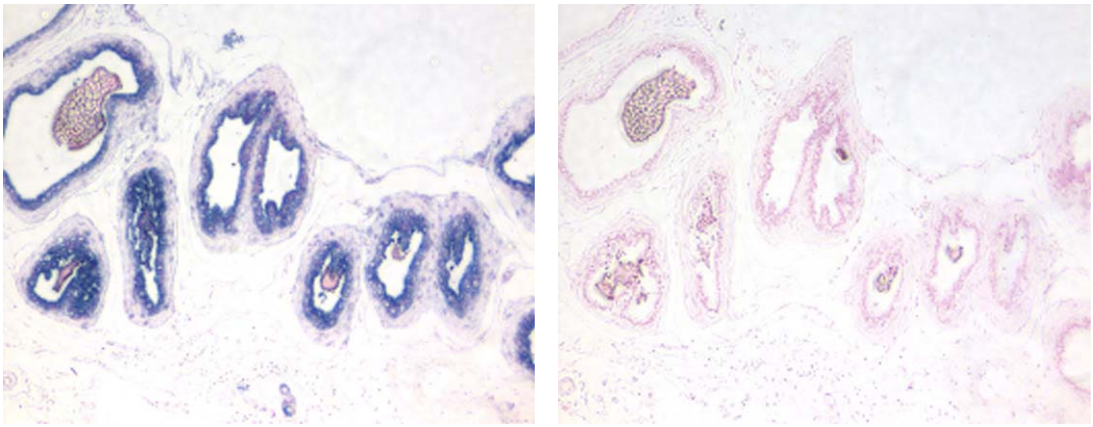

Figure 6: Expression of middle T antigen, detected by *in situ* hybridization, in the dorsal prostate adjacent to the hemagioma found in PSCA-tva mouse injected with middle T antigen. Antisense (left panel); sense control (right panel).

To overcome the problems mentioned above, we moved on to other oncogenes and combinations of oncogenes, which would be unlikely to cause an effect secondary to any low level background. Mice were innoculated with either c-myc virus (n=15), or activated Akt (n=10), or both (n=8). The c-myc group was harvested at one year post injection and examined histologically. Mild hyperplasia was observed in 2 out of 15 mice (Figure 7), suggesting that just like middle T, expressing a single oncogene in a subset of cells in the prostate may not be enough to cause transformation. The Akt group did not show any difference, while the combination group also exhibited mild hyperplasia.

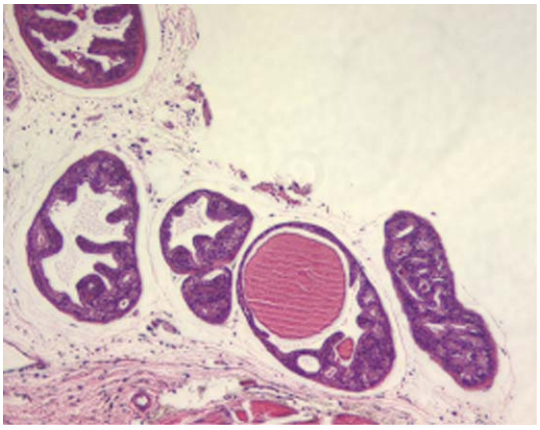

Figure 7: Mild hyperplasia in prostate gland of PSCA-tva mice injected with myc oncogene at one year post injection.

Task 4. As planned in our study, PSCA-tva mice were crossed with Nkx3.1 knockout. This knockout model was chosen over other more aggressive models (for example, PTEN knockout) because we want to see the effects of the introduced genes. We inoculated the bigenic mice (PSCA-tva / NKX3.1 knockout) with c-myc (n=15), activated akt (n=10), and both (n=8). These groups were monitored and their prostates harvested at 1 year after injection. We did not find more evidence of abnormalities other than mild hyperplasia in all groups of mice. As an alternative approach, PSCA-tva mice were crossed with PTEN flox mice and the bigenic offsprings were innoculated with cre-expressing virus to inactivate PTEN in TVA-positive cells. These mice are still being harvested for final analysis, however initial observation at 6 months after injection revealed no obvious abnormalities in the prostate compared to control mice.

KEY RESEARCH ACCOMPLISHMENTS

- Generation of PSCA-tva transgenic mice.
- Successful orthotopic introduction of GFP and luciferase virus into the prostate, with ability to image the prostate.
- In vitro cultivation of the mouse prostate, demonstration of TVA expression, and characterization of this cell population.
- Reconstitution of PSCA-tva prostate epithelium with mesenchyme in mouse recombination model system.
- Inoculation of PSCA-tva mice with middle T virus and generation of hemangiomas
- Strategies to overcome the potential leakiness of the system as suggested by the middle T experiments.
- Combination strategies for better gene delivery: both IP and orthotopic routes.
- Demonstration of long term stable expression of delivered genes.
- Delivery of other potential oncogenes to PSCA-tva mice (c-myc and activated Akt).
- Observation that c-myc caused mild hyperplasia.
- Generation of bigenic mice: PSCA-tva / NKX3.1 knockout, and PSCA-tva / PTEN flox.
- Delivery of oncogenes to bigenic offspring.

- Inoculation of combinatorial genes into bigenic mouse prostates did not cause further abnormalities than mild hyperplasia.

REPORTABLE OUTCOMES

- Abstract presentation:
  Garraway I.P., Tran C.P., Cai K., Yang Y., Loda M., R.E. Reiter. *Mouse models for induction and imaging of bladder neoplasms*. American Urological Association Annual Meeting 2005.
  Garraway I.P., Tran C.P., R.E. Reiter. *Development of a novel mouse model for induction and imaging of bladder cancer***.** American Urological Association, Annual Meeting 2003.

- DOD grant applied by Garraway was funded in 2006, using the transgenic PSCA-tva mouse line to create a new animal model.

CONCLUSION

In the first part of the project we created a mouse model and showed evidence of similarities between the TVA-positive subset of murine cell population and the human PSCA-positive prostate cells. We then proceeded to demonstrate that in this system, virus carrying potential oncogenes can be inoculated into the transgenic mice via orthotopic and intraperitoneal routes, and the delivered genes expressed stably in the prostate over long term. This model was used to introduce c-myc and activated Akt, either alone or in combination, into the TVA-positive cells of the prostate to induce transformation. We also applied this system on the knockout backgrounds of Nkx3.1 or PTEN to study effects on the prostate in the context of tumor suppressor loss. The results showed only mild hyperplasia in the prostate, which implies that the TVA-positive cells are not the optimal targets for transformation in this animal model, and possibly earlier mutations are required to transform the prostate epithelium. Of note however, while observing reports from other groups using this system in other organs, we have realized that the TVA system works best for organs that are solid mass, such as the brain or liver. For our system, the efficiency of infection would be much lower since the mouse prostate is composed of a number of glands, not as single solid mass, therefore it is difficult to infect every gland with one single injection. Overall, the results suggest that introducing potential oncogenes into PSCA positive cells, a late intermediate cell lineage of the prostate epithelium, may not be sufficient to cause cancer. Furthermore, this implies that the process of transformation may require genetic alteration in an earlier, less differentiated subset of cells in the prostate epithelium.